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Continuous anaerobic digestion of swine manure: ADM1-based modelling and effect of addition of swine manure fibers pretreated with aqueous ammonia soaking (AAS)

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Abstract

Anaerobic digestion of manure fibers presents challenges due to their low biodegradability. Aqueous ammonia soaking (AAS) has been tested as a simple method to disrupt the lignocellulose and increase the methane yield of manure fibers. In the present study, mesophilic anaerobic digestion of AAS pretreated manure fibers was performed in CSTR-type digesters, fed with swine manure and/or a mixture of swine manure and AAS pretreated manure fibers (at a total solids based ratio of 0.52 manure per 0.48 fibers). Two different simulations were performed. In the first place, the Anaerobic Digestion Model 1 (ADM1) was fitted to a manure-fed, CSTR-type digester and validated by simulating the performance of a second reactor digesting manure. It was shown that disintegration and hydrolysis of the solid matter of manure was such a slow process that the organic particulate matter did not significantly contribute to the methane production. In the second place, ADM 1 was used to describe biogas production from the codigestion of manure and AAS pretreated manure fibers. The model predictions regarding biogas production and methane content were in good agreement with the experimental data. It was shown that, AAS treatment significantly increased the disintegration and hydrolysis rate of the carbohydrate compounds of the fibers. The effect of the addition of AAS treated fibers on the kinetics of the conversion of other key compounds such as volatile fatty acids was negligible.

Keywords: ADM1; anaerobic digestion; aqueous ammonia soaking; pretreatment; modelling; manure fibers; validation.

1. Introduction

Biogas production based on animal wastes is a common practice, especially in Denmark where a whole biogas industry has been developed based on the co-digestion of manure and industrial waste. In 2011, 24 centralized biogas plants and 60 farm plants based on manure were in operation [1]. Danish biogas plants currently contribute with only 4 PJ in the Danish energy system while the actual energy potential is estimated to be as high as 40 PJ. [2]

This co-digestion is done to increase the methane efficiency and thus the process profitability [3] and the industrial wastes used are characterized by high methane potential, such as slaughterhouse wastes, glycerine, crops, animal fat, fish oil, etc. However, due to the increased demand for biomass feedstock in the bioenergy sector, the prices of these additional biomasses have increased significantly affecting negatively the economy of the biogas sector. This development has triggered the evaluation of novel biomasses such as microalgae [4] or sewage sludge [5] for their potential to be co-digested with manure producing encouraging results but with significant lower methane potential compared to the so far used additional biomasses.

On the other hand, anaerobic digestion (AD) of swine manure alone presents certain challenges, mainly due to the relatively low organic content and the poor biodegradability of the contained solids (i.e. rigid lignocellulosic structured manure fibers), which render the process economically unfavourable [6] In the effort to increase the methane potential of the manure, many different strategies have been investigated so far. New separation technologies, to be applied before anaerobic digestion, have been tested during the last few years [7] in

order to reduce the water content of slurries and, thus, facilitate the transport of lower volumes with higher solids content, which can potentially lead to an increase of biogas production per unit volume [8] Additionally, several different pretreatment technologies have been studied, in order to increase the methane yield of manure and manure fibers [9, 10]. These strategies have the ultimate objective of improving the efficiency of the hydrolysis step, which is known to be the rate limiting step of the anaerobic digestion of lignocellulosic materials. [11]

A chemical pretreatment, which in the past has been studied mainly for biological production of ethanol and also for organic acids production, is Aqueous Ammonia Soaking (AAS) [12, 13, 14]. Due to its high volatility, ammonia can be easily recovered and recycled avoiding [15] thus the need for any further consumption of chemicals. In addition, ammonia is quite safe to handle, non-polluting and non-corrosive [15]. Recently, Jurado et al. [16] reported an increase of up to 178 % in methane yield from swine manure fibers, when AAS for 3 days at 20°C was applied before anaerobic digestion in batch methane potential tests. In the present study, the effect of AAS on increasing methane production from swine manure fibers was assessed in continuous digestion experiments.

As AD of different kind of biomasses (i.e. organic wastes, agricultural residues) is increasingly applied for the production of biogas as a renewable energy resource [17], the need for process optimization based on reliable mathematical models becomes necessary [18]. Mathematical models enable the simulation and the representation of the main aspects of a biological system allowing the formulation and validation of some hypothesis and thus improving the understanding of the system [19]. The development of dynamic models can facilitate the automated digester control, eventually resulting to higher process efficiency. In addition, these models may be used for the designing and scale-up of processes, predicting the

system's behaviour under different conditions, reducing the requirements for time consuming collection of experimental results [19] and helping to easier evaluate the economics of AD applications [20].

AD process is characterized by high complexity and non-linearity and by a difficulty to collect adequate amounts of experimental data required for modelling purposes [19]. Therefore, the creation of a model which can be adapted to different biomasses is a very challenging task. Numerous anaerobic digestion models were developed during the last 40 years [21, 22]. The most recent one in the field is the Anaerobic Digestion Model No1 (ADM1), developed in 1998- 2001 and made publicly available in 2002 [23].

ADM1 is a common platform, combining 19 biochemical and 2 physicochemical processes to simulate the behaviour of various components in anaerobic digesters. The ADM1 model includes the major processes that are involved in the bioconversion of complex organic substrates into methane, carbon dioxide and inert byproducts, with the main metabolic intermediates being hydrogen, acetic, propionic and butyric acids. Also, it includes the mass transfer phenomena between liquid to gas phase [23]. The degradation of complex organic material is assumed to pass through four stages: disintegration-hydrolysis, acidogenesis, acetogenesis and methanogenesis [23].

Although the ADM1 was principally developed for anaerobic digestion of sewage sludge, its structure allows modelling of anaerobic degradation for different feedstocks and a number of such ADM1 applications have already been reported in the literature using different feedstock such as lignocellulosic materials or olive pulp [18, 20, 24, 25, 26] including co- digestion processes [27] . However, ADM1 applications on manure-based digesters are scarce in the international literature, with only few studies such as Gali et al. [28], Astals et al. [29] and Girault et al. [30] focusing on swine manure. In most of the cases found in the literature, the

ADM1 is calibrated to the specific substrate(s) composition and processes configuration and afterwards it is validated by predicting actual experimental data. The calibration is performed by selecting the optimal values of some of the kinetic parameters that minimise the differences between model predictions and experimental results (model fitting) and the validation follows by comparing the predictions of the calibrated model to different (than those used for the calibration) experimental results [18, 25, 26, 28, 30, 35, 37, 38].

In the current study, ADM1 was modified and used for the simulation of continuous digestion of liquid swine manure or liquid manure mixed with swine manure fibers. Initially, ADM1 was fitted to the data from a swine manure-fed, continuous anaerobic digester and it was validated by simulating the performance of a second continuous reactor also digesting manure. Subsequently, ADM1 was fitted to the data from a mesophilic continuous reactor fed with a mixture of manure and AAS pretreated swine manure fibers (at a total solids based ratio of 0.52 manure per 0.48 fibers) and it was validated by simulating the performance of a second and third reactor digesting different manure collections at different values of the Hydraulic Retention Time (HRT).

Materials and methods

2.1. Feedstock

The two different batches of swine manure (manure-I and manure-II) and the manure fibers used in the present study were collected at Morsø BioEnergi, Denmark (a mesophilic biogas plant) and stored at -20^{o}C until used. Manure fibers were produced on pig-farms after separation of liquid manure using a decanter centrifuge.

2.2. Analytical methods

125 Determination of total (TS), volatile (VS), total suspended (TSS) and volatile suspended
126 (VSS) solids was carried out according to Standard Methods [31]. Characterization of the
127 liquid fraction of manure and manure fibers included the determination of the soluble
128 components and was done after centrifugation of the samples at 10000 rpm for 10 min and
129 filtration of the supernatant through 0.2 μm membrane filters. Determination of the total
130 concentration of different components of manure fibers was done after drying the fibers at
131 42°C overnight and powdering them.
132 Total and soluble Chemical Oxygen Demand (COD) were measured with Hach Lange kits
133 LCK-914 and LCK-514, respectively.
134 For the determination of non-soluble and soluble organic phosphorus, persulphate digestion
135 and subsequent ascorbic acid photometric determination was applied to the solid and liquid
136 fractions according to APHA 2005 [31]. For the determination of non-soluble and soluble
137 organic nitrogen, digestion with a micro-Kjeldahl apparatus (Büchi SpeedDigester K-436 and
138 Büchi Scrubber B-414) was applied to the solid and liquid samples, followed by distillation
139 (Büchi Distillation Unit K-350) and, finally, titration with 0.01 mole L^{-1} H_2SO_4, for the
140 determination of ammonium ions concentration [31]. The inorganic forms of phosphorus
141 (PO_4^{-3}-P) and nitrogen (NH_4-N) were determined by applying ascorbic acid photometric
142 determination [31] and by using a Hach Lange Kit LCK-305 (1-12 mg L^{-1} range),
143 respectively.
144 Two groups of carbohydrates were determined in manure and manure fibers: the first group
145 was the total carbohydrates (polymers, oligomers and monomers), including those bound in
146 the lignocellulosic biomass and the second group was the simple sugars (only monomers).
147 Analysis of the total carbohydrates was based on the NREL analytical procedure [32]. For the
148 determination of total carbohydrates the samples were dried at 42°C overnight and powdered,

149 while for the determination of simple sugars the samples were centrifuged at 10000 rpm and
150 the supernatant liquid was passed through 0.2 μm pore size membrane filters. Detection and
151 quantification of sugar monomers (glucose, xylose and arabinose) was made with HPLC-RI
152 (Dionex), equipped with an Aminex HPX-87H column (BioRad) at 60°C. A solution of 4
153 mmol L^{-1} H_2SO_4 was used as eluent, at a flow rate of 0.6 mL min^{-1}. Samples for HPLC
154 analysis were acidified with 1 g L^{-1} H_2SO_4 solution, centrifuged at 10000 rpm for 10 min and,
155 finally, filtered through a 0.45μm membrane filter.
156 For the quantification of volatile fatty acids (VFA), samples were centrifuged at 10000 rpm
157 for 10 min and the supernatant liquid was acidified (pH 2-3) with a H_3PO_4 solution (17%
158 w/v), centrifuged again at 10000 rpm for 10 min and filtered through minisart high flow filters
159 (pore size 0.45 μm). VFAs were analysed with a gas chromatograph (PerkinElmer, Clarus
160 400) equipped with a flame ionization detector at 230^{o}C and a capillary column (Agilent HP-
161 FFAP, 30 m long, 0.53 mm inner diameter) with a gradient temperature program from 105^{o}C
162 to 230^{o}C. Nitrogen was used as carrier gas.
163 The biogas production rate was measured by using a gas measuring system (with an estimated
164 measuring uncertainty of ± 3%), based on displacement of parafin oil. Biogas methane
165 content was determined with a gas chromatograph (SRI GC model 310), equipped with a
166 thermal conductivity detector and a packed column (Porapak-Q, length 1.82 m and inner
167 diameter 2.1 mm). The temperature for injector, column and detector was set to 80°C and
168 nitrogen was used as carrier gas. Finally, pH using a WTW inoLab pH 720 pH-meter and
169 alkalinity was determined according to Standard Methods [31].

170 **2.3. Experimental methods**

171 *2.3.1. Pretreatment*

172 Aqueous ammonia solution 32% w/w was used as ammonia reagent for the AAS
173 pretreatment. Samples of manure fibers were soaked in ammonia reagent with a ratio of 10 ml
174 reagent per 1 g TS. The pretreatment conditions were 3 days duration at 22°C in closed glass
175 flasks to avoid ammonia evaporation. After the completion of the pretreatment, water was
176 added, at a ratio of 10 ml per g TS, in order to facilitate the subsequent ammonia distillation
177 step. Vacuum distillation was performed using a rotary evaporator (Buchi RII Rotavapor with
178 a vertical condenser) under 320 mbar and gradually increased temperature of the water bath
179 from 40 to, 60, 80 and finally 90°C. Distillation's duration was 10 and 20 min at the first two
180 and last two temperature levels, respectively.

181 *2.3.2. Continuous experiments*

182 Three mesophilic (38°C) CSTR-type digesters (of 3 L useful volume each) were operated
183 during this study. Reactor-1 was fed with swine manure-I at a hydraulic retention time (HRT)
184 of 25 days. Reactor-2 was initially started-up and reached steady state with manure-I at an
185 HRT of 25 days (phase A). After 110 days, the manure feeding was replaced (phase B) with a
186 mixture of swine manure-I and AAS-treated manure fibers (at a TS based ratio of 0.52
187 manure per 0.48 fibers). Subsequently and after the digester reached a new steady state
188 (second for Reactor-2), the manure used for the feeding mixture was changed from manure-I
189 to manure-II which was characterized by lower TS content (1.53 g TS per 100 g manure-II
190 compared to 3.78 g TS per 100 g manure-I). Reactor-2 was allowed to reach a new steady
191 state (third for Reactor-2) fed with manure-II mixed with AAS-treated manure fibers (always
192 at a TS ratio of 0.52 manure per 0.48 fibers) (phase C) at a HRT of 25 days (Table 1).

193 The feeding of the digesters was intermittent and repeated once a day. The digesters'
194 performance was followed by a daily recording of biogas production and a weekly

195 measurement of biogas methane content and pH and concentrations of volatile fatty acids and
196 soluble COD. When Reactor-1 and Reactor-2 reached steady state (in Reactor-2, at phase B)
197 complete characterisation was made in terms of measurable components which are variables
198 of ADM1 (see paragraph 2.4.2). Subsequently, the digesters were subjected to impulse
199 disturbances of acetic, propionic and butyric acids and soluble fraction of the manure influent
200 (obtained after centrifugation and filtration) in order to study the dynamics of the individual
201 stages of the anaerobic digestion process (i.e. disintegration-hydrolysis, acidogenesis,
202 acetogenesis and methanogenesis). The impulse disturbances were performed as described in
203 the following: 50 ml of sodium acetate, sodium propionate and sodium butyrate (from Sigma-
204 Aldrich) water solutions (at concentrations of 41, 20.6 and 22 g/L, respectively) and around
205 200 ml of the soluble fraction of the influent were consecutively injected into the digester in
206 order to increase the concentration of the injected substrate in the reactor to around 1600,
207 1200 and 1600 mg COD L^{-1} for the acetic, propionic and butyric acids, respectively, and to
208 around 400 mg COD L^{-1} for the soluble fraction [25]. The response of the bioreactors to each
209 one of the above separate impulses was monitored through measurements of biogas
210 production, biogas composition in methane, VFA concentration and soluble COD
211 concentration until all components approach their levels before the impulse.
212 Finally, a third identical reactor (Reactor-3) was started up and was fed with manure-II (phase
213 D) at 25 HRT and after 160 days the manure feeding was replaced (phase E) with a mixture of
214 manure-II and AAS manure fibers (also at a TS ratio of 0.52 manure per 0.48 fibers) and the
215 HRT was reduced to 17 days. Reactor-3 was allowed to reach a steady state at the operational
216 phases D and E as well (table 1).

217 **Table 1 here**

2.4. Modelling

ADM1 was first fitted (manure-based model) to the experimental data obtained from Reactor-1 fed with manure-I in order to estimate the hydrolysis kinetic parameters of carbohydrates, proteins and lipids and the maximum uptake rates of the long chain fatty acids as well as butyric acid, propionic acid and acetic acid using non-linear parameter estimation. The values suggested by Batstone, 2002 [23] were used for the remaining kinetic parameters and all stoichiometric coefficients. The VFA and soluble COD impulses were simulated by a triangular pulse with a duration of 0.02 d. The software used was Aquasim 2.1 and the optimal values of the kinetic parameters were estimated using the secant method. The algorithm resulted in the optimal values of the kinetic parameters by minimizing the sum of the squares of the deviations between experimental measurements and model predicted values for all variables specified as fit targets (i.e. biogas production and methane content, pH, acetate, propionate and butyrate). The deviations were defined globally for all data points and the sum of squares extended over all data points of each variable. Due to the possible nonlinearity of the model equations and due to the numerical integration procedure, the sum of squares was minimized numerically. Subsequently, the model with the obtained values of the kinetic parameters was used to simulate the steady state of phase A (Reactor-2 with manure-I as influent) and phase D (Reactor-3 with manure-II as influent).

Afterwards the model was fitted to the experimental data collected throughout phase B (mixture-based model), where a mixture of manure-I and AAS manure fibers was fed to Reactor-2. The aim of the fitting was to recalculate the hydrolysis kinetic parameters of carbohydrates (*khydr_ch*), proteins (*khydr_pr*) and lipids (*khydr_li*). Finally, the experimental results obtained during phase C (Reactor-2 fed with a mixture of fibers and manure-II) as well as during phase E (Reactor-3 fed with a mixture of fibers and manure-II at a reduced HRT of

242 17 d) were used to validate the model after it was fitted to phase B (codigestion of liquid
243 manure and AAS treated fibers).
244
245 *2.4.1. Correction of the HRT in the kinetic model*
246 A part of the effluent pipe of the digesters used in this study was vertical and, due to the
247 intermittent feeding, the solids content of the liquid staying in this vertical part was settling
248 back in the digester when the digesters were not fed. This configuration was equivalent with a
249 CSTR equipped with a solids separator returning part of the solids back in the reactor and thus
250 increasing the solids retention time (SRT) as described by [33]. As a result, there was a slight
251 accumulation of solids, which was quantified by comparing the TSS concentration of the
252 interior and of the effluent of the reactors (indicatively, 37.2 ± 1.1 g/L TSS inside the reactor
253 and 27.3 ± 2.1 g/L TSS in the effluent).
254 For modelling purposes, it was assumed that solids and microbial biomass were recycled in
255 the reactor and their concentration was corrected according to eq. 1 [26]
256

257 $$x' = x \cdot \left(Q_{out} - \frac{V_{reactor}}{tres + \frac{V_{reactor}}{Q_{out}}} \right) \quad (1)$$

258 where x' is the solids and microbial biomass recirculation flow (kg COD d^{-1}), x is the solid
259 and microbial biomass concentration (kg COD m^{-3}), $V_{reactor}$ is the reactor active volume (in
260 m^3) and Q_{out} is the effluent flow (m^3 d^{-1}). The higher retention time of the solids was
261 expressed by introducing a new variable into the model, called '*tres*'. *Tres* was defined as the
262 difference between HRT and SRT:

263 *tres* = SRT – HRT

264

265 *2.4.2. Organic and inorganic inputs in the kinetic model.*

266 Swine manure and swine manure fibers were characterized in terms of pH, TS and VS, total
267 and soluble COD, total carbohydrates and free sugars, total and soluble Kjeldahl nitrogen and
268 NH_3-N, volatile fatty acids (valeric, butyric, propionic and acetic acids), inorganic phosphorus
269 and alkalinity. Particulate and soluble inerts were determined as the residual COD after three
270 months of batch anaerobic digestion. Other substances, that are included in ADM1 but were
271 not directly measured, were estimated as follows:
272 Particulate carbohydrates and proteins were assumed to be the difference between total
273 carbohydrates and free sugars and total and soluble Kjedahl nitrogen, respectively.
274 Aminoacids were assumed to be the difference between soluble Kjendahl nitrogen and NH_3-
275 N. Particulate lipids were assumed to be the difference between non-soluble COD (total –
276 soluble COD) and the sum of particulate carbohydrates, proteins and inerts, while long-chain
277 fatty acids were assumed to be the difference between soluble COD and all measured soluble
278 components (sugars, aminoacids, volatile fatty acids and soluble inerts). Total inorganic
279 carbon was directly related to the alkalinity measurement, after correcting for the free
280 ammonia content, at the respective pH. The difference of cations and anions concentration for
281 ions other than the measured ones in the manure was calculated applying the charge balance
282 equation as described by Batstone . [23].

283 $$\sum S_{C^+} - \sum S_{A^-} = 0 \qquad (3)$$

284 Where $\sum S_{C^+}$ represents the total cationic equivalents' concentration and $\sum S_{A^-}$ the total

285 anionic equivalents' concentration.

286 As it has been done in previous studies [34, 35], the solid matrix was assumed to consist of
287 carbohydrates, proteins, lipids and particulate inerts and the hydrolysis was considered as the
288 first step towards methanogenesis. This approach has recently been suggested as most
289 appropriate due to the uncertainty with composite analysis as well as with distinguishing
290 disintegration from hydrolysis parameters [36]. It has to be noted however, that the
291 disintegration parameter was assumed constant (with its value taken from Batstone 2002 [23])
292 and thus the hydrolysis kinetic parameters estimated in the present study incorporated
293 disintegration parameters as well. Consequently, the estimated hydrolysis parameters were
294 actually the apparent hydrolysis parameters and any differences in the apparent hydrolysis
295 kinetic parameters (e.g. due to AAS pre-treatment of fibers) should be attributed also to the
296 disintegration step rather than to only the hydrolysis step. As it has been shown by Jurado et
297 al [16], AAS directly affects the disintegration step and thus releases carbohydrates, which
298 can be further hydrolysed, from the lignocellulosic matrix.

299

300 *2.4.3. Reactor input expressions*

301 In the model, the influent concentration (*influent_C_x*) of a component (*x*) was expressed in
302 kg COD·(100 kg TS)$^{-1}$ for organics or kmol·(100 kg TS)$^{-1}$ for inorganics. This was multiplied
303 with the influent TS concentration (*influent_C_TS*) in kg m^{-3} and thus, the influent
304 concentration of each component *x*, (*influent_C_x_vol*) was calculated (in kg COD m^{-3} and
305 kmol m^{-3} for organic and inorganic components, respectively), according to eq. 4:

306 $$\text{influent\_C\_x\_vol} = \text{influent\_C\_x} \cdot \text{influent\_C\_TS} \quad (4)$$

307 This formulation allows the model to take into account the variations of the TS concentration

in the feeding. The reactor input rate, *input_C_x* for each individual component *x* (in kg COD d^{-1} and kmol d^{-1} for organic and inorganic substances, respectively) was calculated as the product of the influent concentration in *x* with the influent volumetric flow rate, Q_{in}.(in m^3 d^{-1}) as follows:

$$input\_C\_x = influent\_C\_x\_vol \cdot Q_{in} \quad (5)$$

3. Results and discussion

3.3. Influent characterization

The TS content of manure-I, manure-II and AAS pretreated fibers was 37, 15 and 129 kg m^{-3}, respectively. The TS to VS ratio in the manure-I, manure-II and AAS pretreated fibers was 1.8, 1.7 and 1.26, respectively. Detailed composition of manure and AAS-pretreated fibers is presented in table 2 where it can be seen that there are significant differences between manure-I and manure-II. All components in the soluble fraction had higher concentrations in manure-I than in manure-II. However higher concentrations of particulate matter was found on manure-II. As anticipated, the AAS-pretreated fibers consisted mainly of particulate organic matter, with carbohydrates being a substantial fraction. On the other hand, manure was rich also in soluble organic matter, mainly in long chain and volatile fatty acids.

Table 2 here

2.3.Continuous experiments

Figure 1 shows the volumetric and TS loading rate of the digesters. Operating conditions and different steady-state characteristics of the anaerobic digesters are shown in table 3. Although the operating conditions were very similar for both Reactor-1 and Reactor-2 and despite the

330 fact that manure fibers were characterised by a much lower content in soluble organic
331 material (table 2), the biogas productivity (table 3) of Reactor-2 at Phase B (when digestion of
332 the mixture of manure with AAS-treated raw fibers took place) was 22% higher than that of
333 Reactor-1 and Reactor-2 at phase A (with only manure as influent). The methane yield (table
334 3) was calculated as 0.27 L CH_4 (g TS added)$^{-1}$ for Reactor-2 at the steady state of Phase A
335 (fed only with manure). This result is in accordance with the methane yield reported in
336 previous studies [6]. For Reactor-2 during the steady state at Phase B, when the digester was
337 fed with mixture of manure and AAS-pretreated fibers, the methane yield presented a value of
338 0.25 L CH_4 (g TS added)$^{-1}$. Given that the TS ratio of manure to fibers in the influent of
339 Reactor-2 was 0.52:0.48 and assuming that the methane yield due to the manure fraction was
340 0.27 L CH_4 (g TS)$^{-1}$ also in Phase B of Reactor-2, the methane yield of AAS-pretreated fibers
341 was calculated to be 0.23 L CH_4 (g TS)$^{-1}$. This was confirmed in Reactor-2, phase C where
342 methane yield of AAS manure fibers was similarly calculated to be 0.25 L CH_4 (g TS)$^{-1}$.
343 In previous studies [16], the ultimate (after 50 days) methane yield from swine manure fibers
344 was measured to be in the range of 0.12-0.13 L CH_4 (g TS)$^{-1}$ in batch experiments.
345 Consequently, AAS pretreatment resulted to a 98% increase of the methane yield of manure
346 fibers in continuous anaerobic digestion and at a hydraulic retention time of 25 d. This lies
347 within the range of the results obtained from batch experiments [16] where an increase of
348 180% was observed for a loading of 0.16 g TS per 10 ml of inoculum, while the observed
349 increase was 54% at a higher loading of 0.25 g TS per 10 ml of inoculum [16].

350 **Table 3 here**

351 **Figure 1 here**

352 **2.4.Modelling**

353 At first place, the IWA anaerobic digestion model (ADM1) was fitted to the experimental data
354 obtained from the manure fed Reactor-1 and the calculated kinetic parameters (manure-based
355 model) are shown in Table 4. The parameters estimated were the maximum specific uptake rate
356 for acetic, propionic, butyric and long-chain fatty acids (*km_ac, km_pro, km_c4* and *km_fa* respectively)
357 and the hydrolysis kinetic parameters for carbohydrates, lipids and proteins (*khydr_ch*, *khydr_li* and
358 *khydr_pr* respectively). The difference of cations and anions concentration in the manure was also
359 estimated and found close to the calculated one, as described in section 2.4.2 (0.542 and 0.454
360 kmole m^{-3}, respectively). The estimated values of the hydrolysis parameters (table 4) showed that
361 hydrolysis of the particulate matter is very slow and thus the contribution of the particulate matter
362 to the methane production could be assumed as very limited.

363 **Table 4 here**

364 The quality of the fitting was quite satisfactory as shown in table 5, where the experimental
365 and model predicted characteristics of the steady state of Reactor-1 are shown. Also, the
366 model was fitted quite well to the biogas production and methane content (figure 2) as well as
367 to the acetic, propionic and butyric acid concentrations under impulse disturbances (figure
368 5a).

369 **Table 5 here**

370 **Figure 2 here**

371

372 Subsequently, the fitted manure-based model was used to simulate the performance of

373 Reactor-2 during the two different phases (phase A with only manure-I and phase B with the
374 mixture of manure-I and AAS-treated fibers as influent). Experimental data and model
375 predictions for both steady states are shown in table 6 and figure 3.

376 **Table 6 here**

377 It is obvious that the manure-based model generated from Reactor-1 predicted quite
378 satisfactorily the steady state of Reactor-2 at phase A when it was fed only with manure (0-
379 109 days). However, the model simulated poorly the steady state and biogas production and
380 composition in methane during phase B, when AAS-treated fibers were added to the influent
381 of Reactor-2. Specifically, significantly lower biogas production and higher methane
382 percentage in the biogas was predicted.

383 **Figure 3 here**

384 This could be attributed to the different lipids and carbohydrates content in the manure and
385 AAS pretreated fibers (table 2) in relation to the hardly biodegradable nature of carbohydrates
386 found in manure, which is expected to differ when the solid part has undergone AAS
387 treatment. Anaerobic degradation of lipids results in higher methane content compared to the
388 anaerobic degradation of carbohydrates. The model was adapted to the higher lipids content
389 and the lower (and hardly biodegradable) carbohydrates content of the manure and therefore it
390 overestimated the methane content and underestimated the methane production when the
391 reactor was fed with AAS pretreated fibers (containing less lipids and more biodegradable
392 carbohydrates).
393 In order to account for the difference in carbohydrates biodegradability due to AAS treatment

394 the re-estimation of the hydrolysis kinetic parameters was the next step. In addition, whether
395 the feeding change from manure to a mixture of manure and AAS-treated fibers affected or
396 not the kinetics of consumption of volatile fatty acids was investigated.
397 Therefore, a new fitting of the ADM1 hydrolysis parameters was performed using the data
398 obtained from Reactor-2 in phase B (co-digestion of manure-I and AAS pretreated fibers).
399 The kinetic parameters estimated during this new fitting were hydrolysis parameters for
400 carbohydrates, lipids and proteins (*Khyd_ch*, *K_hyd_li* and *K_hyd_pr*, respectively) and their
401 values are shown in table 4 (mixture-based model). As it was expected, hydrolysis parameters
402 (which incorporate hydrolysis and disintegrations step, section 2.4.2) of all lipids, proteins
403 and carbohydrates changed. The change was substantial for carbohydrates which after AAS
404 contributed extensively on methane production. This confirms the results presented by Jurado
405 et al. [16], where the increase of methane yield in batch tests was attributed to the effect of
406 AAS on the disintegration step. After fitting, the model simulated very well the biogas
407 production and methane content, as shown in figure 4 and table 7. It has to be emphasized
408 here, that the apparent hydrolysis kinetic parameters estimated in the present study
409 incorporated disintegration parameters as well. Thus, a comparison with the estimated values
410 of hydrolysis kinetic parameters from previous studies is not valid. While in the most recent
411 studies the hydrolysis kinetic parameters range from 0.1 to 10 d^{-1} [30, 18, 37, 38, 23], in the
412 present study the apparent hydrolysis kinetic parameters estimated are at least two orders of
413 magnitude lower (table 4). Thus, the disintegration of particulate matter is the rate limiting
414 step during the anaerobic digestion of swine manure and manure fibers and it is this step that
415 is mostly affected by the application of AAS as a pretreatment for swine manure fibers.
416 The maximum uptake rates of volatile fatty acids in the mixture based model were kept the
417 same with them in the manure based model and the mixture based model was able to simulate

418 satisfactorily the acetic, propionic and butyric acid concentration profiles during steady state
419 and under impulse disturbances (Reactor-2 in Phase B), as shown in figure 5b. This implies
420 that the maximum uptake rates for the volatile fatty acids were not influenced by the addition
421 of AAS-treated fibers to the influent, something that was anticipated as the reactor was not
422 kinetically limited and the VFAs level was very low.

423 **Figure 5 here**

424 Conclusions

425 In the present study, the efficiency of AAS as a treatment method for increasing the methane
426 yield of manure fibers has been verified in continuous digesters. The performance of
427 mesophilic CSTR-type anaerobic digesters, fed with mixtures of swine manure and AAS-
428 treated manure fibers (at a TS based ratio of 0.52 manure per 0.48 fibers) was assessed in
429 comparison to a similar digester fed only with manure. Mixing of AAS-treated manure fibers
430 with the influent manure resulted at an increase of 22 % in biogas productivity and 98% in
431 methane yield of the fibers (compared to manure fibers without treatment) in continuous
432 anaerobic digestion at a hydraulic retention time of around 25 d.
433 The Anaerobic Digestion Model 1, ADM1, was fitted to a manure-fed, continuous anaerobic
434 digester and validated by simulating the performance of a second also continuous anaerobic
435 digester digesting manure. It was shown that disintegration and hydrolysis of the solid matrix
436 of swine manure was an extremely slow process, in the degree that organic solids did not
437 actually contribute to the methane production. In a second stage, ADM1 was fitted to a
438 mesophilic continuous reactor running with a mixture of manure and AAS pretreated swine
439 fibers (at a TS based ratio of 0.52 manure per 0.48 fibers). It was shown that, in this case, the

disintegration/hydrolysis of carbohydrates contributed substantially to the methane production proving that AAS treatment significantly increased the disintegration/hydrolysis rate of the carbohydrate compounds of the fibers. Furthermore, the addition of AAS-treated fibers to the influent did not significantly affect the kinetics of consumption of volatile fatty acids.

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554 **List of tables**

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595 **Table 1.** Characteristics of the different experimental phases in the three reactors

	Phase	Feedstock	Ratio (TS basis)	HRT	Uses in ADM1
Reactor-1		Manure-I		25	Fitting manure-based model
Reactor-2	A	Manure-I		25	Validation manure-based model
	B	Manure-I + AAS fibers	0.52:0.48	25	Fitting mixture-based model
	C	Manure-II + AAS fibers	0.52:0.48	25	Validation mixture-based model
Reactor-3	D	Manure-II		25	Validation manure-based model
	E	Manure-II + AAS fibers	0.52:0.48	17	Validation mixture-based model

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Table 2. Characteristics of manure-I and manure-II and of AAS-pretreated manure fibers

Characteristics, particulate matter	Manure-I (Used in phases A and B)	AAS-pretreated raw fibers (Used in phases B, C and E)	Manure-II (Used in phases C, D and E)
COD, kg / 100 kg TS	79.11 ± 8.1	98.6 ± 9.4	124.19
Carbohydrates, kg COD/ 100 kg TS	6.10 ± 0.11	47.79 ± 1.09	9.57[a]
Proteins, kg COD/ 100 kg TS	28.00 ± 1.40	20.12 ± 0.98	43.95[a]
Lipids, kg COD/ 100 kg TS	32.65 ± 1.01	14.58 ± 0.52	51.25[a]
Inerts, kg COD/ 100 kg TS	12.37 ± 0.82	16.08 ± 0.74	19.42[a]
Characteristics, soluble matter			
COD, kg / 100 kgTS	91.83 ± 9.90	8.10 ± 0.2	55.90
Sugars, kg COD/ 100 kg TS	0	0	0.0[a]
Aminoacids, kg COD/ 100 kg TS	18.92 ± 0.57	0.21 ± 0.01	11.52[a]
Long chain fatty acids, kg COD/ 100 kg TS	21.25 ± 0.32	3.89 ± 0.61	12.94[a]
Valeric acid, kg COD/ 100 kg TS	0	0.11 ± 0.10	0.0[a]
Butyric acid, kg COD/ 100 kg TS	3.98 ± 0.02	0.16 ± 0.80	2.42[a]
Propionic acid, kg COD/ 100 kg TS	8.46 ± 0.03	0.34 ± 0.20	5.15[a]
Acetic acid, kg COD/ 100 kg TS	34.75 ± 0.18	2.07 ± 0.15	21.15[a]
Inerts, kg COD/ 100 kg TS	4.46 ± 0.10	1.34 ± 0.08	2.71[a]
Inorganic carbon, kmole / 100 kg TS	0.53 ± 0.00	0.099 ± 0.002	0.78
Inorganic phosphorus, kmole / 100 kg TS	$4.73x10^{-6}$± $0.31x10^{-3}$	$4.77x10^{-3}$ ± $0.30x10^{-3}$	$2.548x10^{-3}$
Inorganic nitrogen (NH_3-N), kmole / 100 kg TS	0.73 ± 0.02	$8.63x10^{-3}$± $0.25x10^{-3}$	1,05
Difference of cations and anions concentration, kmole (100 kg TS)$^{-1}$	0.454	0.14	0,128

[a] characteristics calculated based on the TS content, non-soluble (particulate) and soluble COD and the distribution of the particulate and soluble COD according to manure-I characteristics

Table 3. Operating conditions and steady state characteristics for the different phases of the continuous anaerobic digestion

Operating Conditions	**Reactor-1**	**Reactor-2**			**Reactor-3**	
		Phase A	**Phase B**	**Phase C**	**Phase D**	**Phase E**
Feeding	**Manure-I**	**Manure-I**	**Manure-I + AAS fibers**	**Manure-II + AAS fibers**	**Manure-II**	**Manure-II + AAS fibers**
Hydraulic Retention Time (d)	26.0 ± 3.5	26.7 ± 1.9	23.0 ± 3.4	25.7±1.6	28.34 ± 2.8	16.3± 2.6
Influent total COD (g L^{-1})	71.1 ± 13.1	72.1 ± 17.0	69.8 ± 4.9	-	25.96	25.4±0.7
Influent soluble COD (g L^{-1})	33.4 ± 3.6	35.4 ± 1.0	27.9 ± 0.2	-	9.8	3.28±0.05
Characteristics at steady state						
Biogas productivity (L L^{-1} d^{-1})	0.86 ± 0.15	0.86 ± 0.11	1.05 ± 0.09	0.35±0.05	0.166± 0.02	0.52±0.08
Methane (%)	60.9 ± 3.0	61.7 ± 4.2	61.2 ± 2.6	62.9	64.2 ± 2.9	64.7±3.2
Methane yield (L g^{-1} COD added)	0.19 ± 0.05	0.20 ± 0.06	0.21 ± 0.04	0.22±0.04	0.11±0.02	0.21±0.04
Methane yield (L g^{-1} TS added)	0.28 ± 0.08	0.27 ± 0.06	0.25 ± 0.05	0.19±0.07	0.13 ± 0.02	0.19±0.01
Volatile Fatty Acids (mg L^{-1})	160 ± 5	165 ± 6	170 ± 4		150	
pH	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.05±0.17	8.04± 0.01	7.9±0.22
Effluent soluble COD (g L^{-1})	5.4 ± 0.7	6.0 ± 0.4	4.8 ± 0.9		1.7± 0.1	2.26±0.5
TS inside reactor (kg m^{-3})	46.7± 1.5	49.8± 7.8	46.9± 1.8		23.17± 1.6	46.82± 6.9
VS inside reactor (kg m^{-3})	21.92± 1.02	29.4± 5.3	25.5± 0.78		11.8± 1.4	21.67± 4.22

Table 4. Estimated values of the kinetic parameters and difference of cations and anions concentration after fitting of the ADM1 to the experimental data from Reactor-1 (manure fed reactor) and Reactor-2 (fed with mixture of manure and AAS_fibers).

Kinetic parameter	**Units**	**Manure-based model**	**Mixture-based model**
Carbohydrates hydrolysis, *khydr_ch*	d^{-1}	<<	7.3×10^{-2}
Proteins hydrolysis, khydr_*pr*	d^{-1}	3.0×10^{-3}	7.1×10^{-3}
Lipids hydrolysis, *khydr_li*	d^{-1}	2.8×10^{-4}	4.2×10^{-3}
Maximum uptake rate of long chain fatty acids, *km_fa*	kg COD fa/kg COD x_fa/d	0.93	0.93
Maximum uptake rate of butyric acid, *km_c4*	kg COD c4/kg COD x_c4/d	13.1	13.1
Maximum uptake rate of propionic acid, *km_pro*	kg COD pro/kg COD x_pro/d	6.56	6.56
Maximum uptake rate of acetic acid, *km_ac*	kg COD ac/kg COD x_ac/d	45.02	45.02
Difference of cations and anions concentration	kmole $(100 \text{ kg TS})^{-1}$	0.54	-

Table 5 Model predictions (using manure-based model) and experimental measurements for the steady state of Reactor-1 after the model fitting and the estimation of the kinetic parameters.

Steady state characteristics	**Reactor-1 (Manure-based model)**	
	Experimental	**Model**
Methane, %	61.0 ± 0.6	64.3 ± 0.1
Biogas production, $m^3\ d^{-1}$	$2.2 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3}$	$2.4 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3}$
pH	8.2 ± 0.1	8.1 ± 0.1
Acetic acid, $kgCOD\ m^{-3}$	0.035 ± 0.014	0.226 ± 0.030
Propionic acid, $kgCOD\ m^{-3}$	0.005 ± 0,002	0.021 ± 0.001
Butyric acid, $kgCOD\ m^{-3}$	0.005 ± 0.003	0.014 ± 0.001

Table 6. Model predictions (using manure based model) and experimental measurements for the steady states of Reactor-2 fed with manure (phase A) and mixture of manure and AAS-treated fibers (phase B).

Steady state characteristics	**Manure (phase A)**		**Mixture of manure and AAS-treated fibers (phase B)**	
	Experimental	**Model**	**Experimental**	**Model**
Methane, %	63.2 ± 1.2	64.3 ± 0.1	62.0 ± 0.0	68.3 ± 0.1
Biogas production, $m^3\ d^{-1}$	$2.2 \cdot 10^{-3} \pm 0.2 \cdot 10^{-3}$	$2.3 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3}$	$3.0 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$	$1.7 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3}$
pH	8.30 ± 0.1	8.20 ± 0.01	8.30 ± 0.02	8.14 ± 0.02
Acetic acid, $kgCOD\ m^{-3}$	0.028 ± 0.007	0.236 ± 0.031	0.028 ± 0.007	0.107 ± 0.017
Propionic acid, $kgCOD\ m^{-3}$	0.003 ± 0.0004	0,021 ± 0,001	Not detected	0.019 ± 0.001
Butyric acid, $kgCOD\ m^{-3}$	0.005 ± 0.004	0.014 ± 0001	Not detected	0.012 ± 0.001

Table 7. Model predictions (using mixture-based model) and experimental measurements for the steady state of Phases C and E after the model fitting and the parameters estimation.

Steady state characteristics	**Mixture of manure and AAS-treated fibers (phase C)**		**Mixture of manure and AAS-treated fibers (phase E)**	
	Experimental	**Model**	**Experimental**	**Model**
Methane, %	62.6 ± 2.1	62.86 ± 0.6	64.9 ± 3.4	65.7 ± 0.9
Biogas production, $m^3\ d^{-1}$	$1.0 \cdot 10^{-3} \pm 1.7 \cdot 10^{-4}$	$9.9 \cdot 10^{-4} \pm 1.8 \cdot 10^{-5}$	$1.4 \cdot 10^{-3} \pm 2.4 \cdot 10^{-4}$	$1.3 \cdot 10^{-3} \pm 6.6 \cdot 10^{-5}$
pH	8.1 ± 0.2	7.8 ± 0.01	7.9 ± 0.2	7.8 ± 0.00